

Esp

Week 9

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

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1 Colony PCR of pSB1C3-T7-EB-LT and Transformation into BL21(DE3)

Responsible

Sigrun

Protocols used

- PCR - Transformation

Modifications and comments to protocols

Normally, colony PCR is performed directly when liquid cultures of TOP10 cells are made. Unfortunately the time was not sufficient to perform the PCR this time so it was performed on plasmid isolated from the liquid TOP10 culture.

Experimental Set Up

Only one colony / liquid culture existed for pSB1C3-T7-EB-LT. 1 μ l of plasmid was used for a PCR reaction of 25 μ l total volume. The isolated plasmid was also directly transformed into BL21(DE3) cells

Results and Conclusions

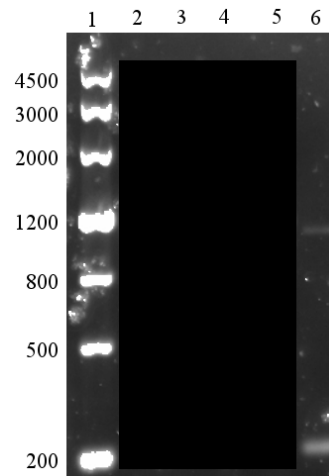


Figure 1: Colony PCR of pSB1C3-T7-EB-LT in lane 6, showing no amplicon of the desired size. Lanes 2 to 5 correspond to an unrelated experiment whose products were run on the same gel.

Discussion and Troubleshooting

The PCR does not seem to have amplified anything for the EB. The assembly might potentially have failed but the PCR product is nevertheless being saved for sequencing and the plasmid was transformed into BL21(DE3) alongside other samples.

The following week, sequencing results arrived and revealed that the plasmid did not contain our sequence of interest.

2 Kirby-Bauer

Responsible

Maren Maania, Oskar Ohman, Sigrun Stulz

Protocols used

- Kirby Bauer

Modifications to protocols

Kirby-Bauer tests can be performed on a variety of agar plates but in this case MacConkey Agar was used to allow for better visibility of the colonies.

Experimental Set Up

The whole experiment was performed with two different protein samples: - EB, expressed on week, labeled 'EB old' 6*

- EB, expressed on week 8, labeled 'EB new'

*The EB samples from the expression in week 6 had been frozen in the lysis buffer and subjected to several freeze-thaw cycles in order to perform SDS-Page. Since this might affect the activity of the protein, a newer sample was chosen for this experiment as well. Samples:

- EB induced with 0.5mM, soluble fraction
- EB induced with 0.5mM, insoluble fraction
- EB induced with 1mM, soluble fraction
- EB induced with 1mM, insoluble fraction
- untransformed cells, soluble fraction
- untransformed cells, insoluble fraction
- Kanamycin (35 $\mu\text{g}/\text{l}$)

Briefly, autoclaved filter paper was soaked in 50 μl of sample for 10 minutes. 200 μl of Tob1 E.coli were streaked onto each plate and left to dry briefly. The filter paper was placed onto the plate and placed in the incubator at 37C overnight.

Results and Conclusions

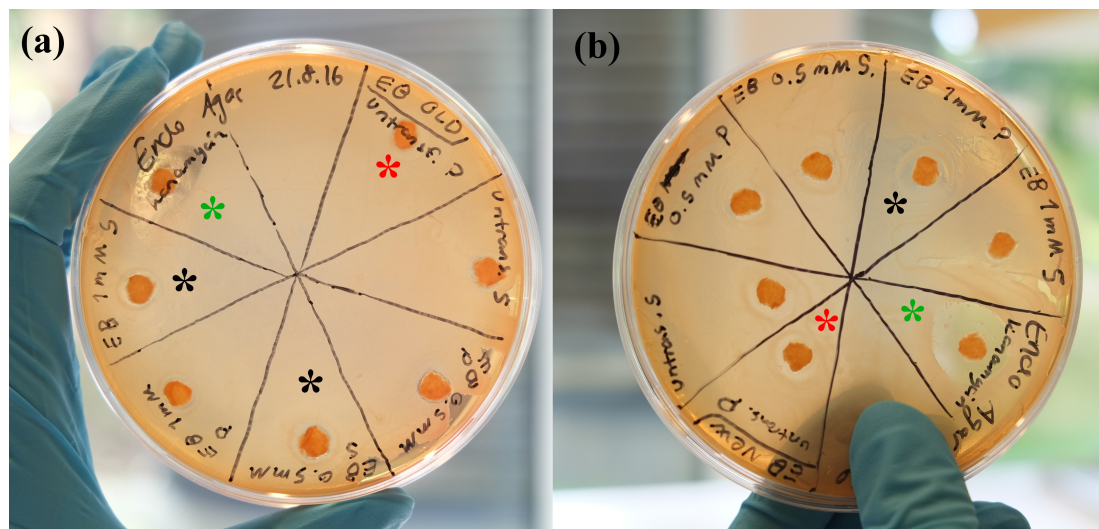


Figure 2: Kirby-Bauer of (a) 'old' EB and (b) 'new' EB. Green asterisk marks positive control, black asterisks any sample exhibiting halo formation and red asterisk the corresponding negative control.

Table 1: compiled Results from Kirby-Bauer

Protein	Sample with halo	Comments
EB old	1mM IPTG induction, soluble fraction	halo even for negative control
	0.5 mM IPTG induction, soluble fraction	
EB new	1mM IPTG induction, insoluble fraction	

Discussion and Troubleshooting

The results for this experiment are not conclusive - different samples showed halo formation when protein expressed on different dates was used (EB 'old' and 'new') and whereas there seems to be a pattern that samples produced under higher IPTG induction are more active, it remains unclear whether EB is present in the soluble or insoluble fraction. The inconclusive results could be due to a variety of factors, chief among them that EB might simply not display bacteriolytic activity. Whereas it has been shown that Esp efficiently degrades biofilm, any bacteriolytic or bacteriostatic activity has, to our knowledge, not been investigated.

Despite the fact that EB might simply not display an activity that could be measured by a simple Kirby-Bauer, we decided to repeat the experiment and optimise the set-up to get more meaningful data out of it. In this first experiment, the filter paper that was used was not ideal and did not seem to soak up the protein solution very well. This perhaps also led to excess liquid clinging to the filter paper, which in itself could lead to a halo formation without the protein having any direct function in it. This can be noticed on the 'EB new' plate quite well,

where even cell lysates from untransformed cells showed a quite considerable halo formation. Additionally, more equal streaking of the plate should be attempted since on some of the plates' edges it appears as if the bacterial load is uneven.

3 Protein Expression pSB1K3-T7-EB

Responsible

Shuangjia Xue, Sigrun Stulz

Protocols used

- Protein Expression

Experimental Set Up

The usual IPTG concentrations of 0.5mM and 1mM were used and samples were collected after 4h. They were stored overnight at 4C to avoid repeated freeze-thaw cycles.

Results and Conclusions

No results at this stage.

Discussion and Troubleshooting

The freshly produced protein can be used to perform activity tests as well as an SDS-Page.

4 Transformation of pSB1C3-T7-trEB into TOP10 and BL21

Responsible

Sigrun Stulz

Protocols used

- Transformation

Modifications to protocols

Since improved transformation rates were reported after letting cells recuperate in 200 μ l SOC for 2 hours instead of in 100 μ l for 1h. This modification was attempted here.

Experimental Set Up

See Modifications

Results and Conclusions

No results at this stage but the following day, no colonies were observed.

Discussion and Troubleshooting

Both transformation and ligation have been proven to be troublesome in our project. In this case, a ligated product could be seen on the gel, making it likely that the issue was in fact with transformation. A re-transformation can be attempted with the remaining ligation product.

5 Competence Test of XL-1 Blue Supercompetent Cells

Responsible

Sigrun Stulz, Oscar He, Ellinor Lindholm

Protocols used

This test was conducted to assess whether some left-over XL1 Blue supercompetent cells from last year could still be used. They had been stored at -20C for over a year, resulting in doubts about their competence.

The manufacturer's protocol was used, accessible here: <http://www.chem-agilent.com/pdf/strata/200249.pdf>

Experimental Set Up

Samples:

- pUC19 (positive control from manufacturer)

- RFP coding construct (from competent cell test kit) at 50 ng/ μ l

- Ligation product pSB1C3-T7-trEB

Results and Conclusions

No results at this stage but the following day no colonies were observed, likely indicating that the cells had lost their competency during the storage time at -20C

Discussion and Troubleshooting

The cells won't be used in any experiments.

6 Overhang PCR on pSB1K3-T7-EB

Responsible

Oscar Ohman, Shuangjia Xue, Sigrun Stulz

Protocols used

- Q5 High Fidelity Master Mix PCR

Modifications to protocols

Reaction was performed in a total volume of 12.5 μ l

Experimental Set Up

This reaction had been performed successfully before but more PCR product was needed to repeat digestion and ligation. 20 ng of template DNA was used to set up the reaction for each EB1 and EB2

Results and Conclusions

The PCR reaction was considered successful and the resulting concentrations were:

EB1: 676.8 ng/ μ l

EB2: 581.5 ng/ μ l

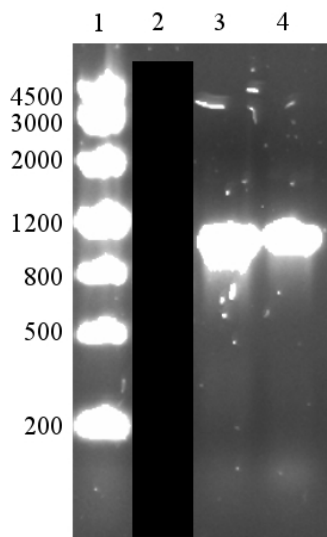


Figure 3: Successful Overhang PCR of EB1 and EB2 can be observed in lanes 3 and 4. Lane 2 corresponds to an unrelated experiment that was run on the same gel

7 Digestion and Ligation of T7-EB-BamHI

Responsible

Oskar hman, Shuangjia Xue, Sigrun Stulz

Protocols used

- 3A Assembly

Modifications to protocols

- For each digestion, 2000 ng of DNA was used.
- In the PCR purification performed after digestion, samples were applied to the column in the binding buffer five times, which has been shown to lead to a higher yield during PCR purification.
- For the ligation, the reactions were scaled down to 30 μ l for EB1 and 25 μ l for EB2 because there wasn't enough backbone left to do two full reactions.
- The vector:insert ration for this ligation was 1:10
- The Ligation was performed at 16C overnight instead of at RT for 10 minutes.

Experimental Set Up

EB1 and EB2 were double digested with EcoRI and BamHI and then PCR purified

EB1 5.2 ng/ μ l

EB2 6.5 ng/ μ l

Previously digested LT and pSB1C3 were used

Results and Conclusions

Table 2: Digestion and Ligation Products

Well	Sample	Expected Size	Conclusion
1	DNA Ladder		
2	pSB1C3-T7-EB-LT (1) Ligation	3002 bp	Ligation largely unsuccessful
3	pSB1C3-T7-EB-LT (2) Ligation	3002 bp	Ligation largely unsuccessful
4	T7-EB-BamHI (1) dd	854 bp	Not assessable*
5	T7-EB-BamHI (2) dd	854 bp	Not assessable*
6	pSB1C3 dd	ca 2070 bp	Not assessable*
7	LT dd	79 bp	Not assessable*

*Those templates have the restriction sites far towards the ends so that only a small number of bases will be cleaved off. That means that successful digestion can normally not be assessed by DNA Gel Electrophoresis.

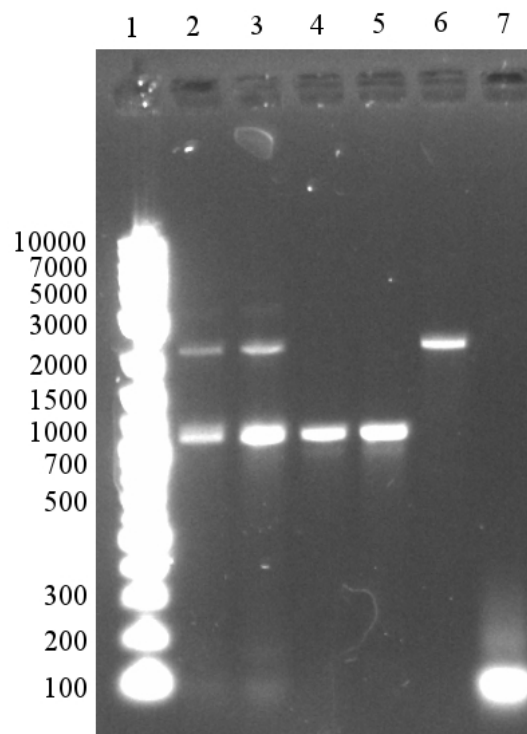


Figure 4: Gel Electrophoresis of digested and ligated fragments

Discussion and Troubleshooting

On this gel we were able to see that our template DNA seems to be present and of good quality. Despite this, the ligation doesn't appear to have been very successful. A small band larger than our longest digestion product can be observed but it is very weak compared to the digestion products. The samples will nevertheless be used for transformation.

If a next ligation is necessary, it could be performed with another backbone.

Kirby-Bauer

Responsible

Reskandi Rudjito, Sigrun Stulz

Protocols used

- Kirby-Bauer

Modifications to protocols

- OD600 of the bacterial inoculum was not assessed before streaking.

Experimental Set Up

Table 3: Samples for Kirby Bauer

Sample	Fraction	Dilution
EB 0.5mM IPTG	soluble	undiluted
EB 0.5mM IPTG	soluble	3x diluted
EB 0.5mM IPTG	insoluble	undiluted
EB 0.5mM IPTG	insoluble	3x diluted
EB 1mM IPTG	soluble	undiluted
EB 1mM IPTG	soluble	3x diluted
EB 1mM IPTG	insoluble	undiluted
EB 1mM IPTG	insoluble	3x diluted
Kanamycin	-	35 $\mu\text{g}/\text{mg}$
ddH2O	-	-

Two types of plates were made in triplicate, always containing six samples, e.g.: - EB 0.5mM IPTG, soluble, undiluted

- EB 0.5mM IPTG, soluble, diluted 3x.
- EB 0.5mM IPTG, insoluble, undiluted
- EB 0.5mM IPTG, insoluble, diluted 3x.
- ddH2O
- Kanamycin

Plates were incubated at 37°C

Results and Conclusions

The plates were incubated checked after 12h, 24h and 36h but halos weren't very visible and colonies weren't dense. It's likely that the bacterial inoculum wasn't dense enough and the OD600 should have been checked before spreading.

Discussion and Troubleshooting

In a next experiment, bacterial inoculum should be checked for its OD600 before streaking. Additionally, another type of agar could be used to make colony formation more easily visible, e.g. endo agar.